IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 7273
KOLOSSOV *et al.*	Art Unit: 1632
Appl. No.: 10/594,188	Examiner: CHEN, Shin Lin
Filed: June 21, 2007	Atty. Docket: 2590.0040002/EJH/SAC
For: **Novel Method for the Preparation of Embryoid Bodies (EBs) and Uses Thereof**	

Declaration Under 37 C.F.R. § 1.132 of Dr. Silke Schwengberg

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, Silke Schwengberg, Ph.D., declare and state that:

1. I am a co-inventor of the above-captioned U.S. patent application number 10/594,188, filed June 21, 2007, entitled, "Novel Method for the Preparation of Embryoid Bodies (EBs) and Uses Thereof."

2. I received my education at the Ruhr University of Bochum, the University of Hannover, and the Hannover Medical School, all in Germany. A copy of my *curriculum vitae* is attached as Exhibit A.

3. I am currently employed at Axiogenesis, the assignee of the above-captioned application. I hold the position of a senior scientist and the head of laboratory. My work involves the study of stem cells, their differentiation, and the use of stem cell derived tissue-specific cells for in vitro assays.

4. I am familiar with the above-identified application and pending claims as well as the June 26, 2009 Office Action.

5. I understand that present claims are toward methods for producing embryoid bodies (EBs) from pluripotent cells by rocking a container containing a liquid single cell suspension culture of pluripotent cells.

6. I understand that the claims have been rejected in the Office Action for, among other things, being obvious over Dang *et al.*, June 20, 2003 (U.S. 2003/0119107 A1) (hereinafter "Dang") in view of several other publications. I have reviewed the Dang reference.

7. The Dang reference is directed toward the improvement of stirred bioreactors and differentiation of cells in stirred cultures to generate embryoid bodies which is very different from the agitation method of the present invention.

8. As discussed in the specification, the agitation method is superior to the stirring culture method for the preparation of embryoid bodies (EBs).

9. In order to demonstrate the advantages of the agitation method over the well-known stirring method for the preparation of EBs, both methods were compared with respect to (i) yield of EB per ml and (ii) yield of differentiated cells (e.g., cardiac cells) per ES cell originally seeded into the differentiation culture.

10. Several reports can be found in the literature about production of EBs using the stirring method (*e.g.*, spinner flask technology). In one of these reports (Hescheler *et. al.*, Methods in Molecular Biology 185 (2001), 169), the spinner flask technique is described in detail and results are presented with regard to the amount of EBs generated by this method and the percentage of EBs exhibiting cardiac differentiation. In the above-mentioned report, differentiation was started using $1x10^7$ ES cells/ml, and around 1000 EBs were generated in

each flask (250 ml culture volume). 90 % of EBs showed spontaneous beating cardiac cells, but the amount of cardiac cells per seeded ES cell was not determined.

11. Using the agitation method described in the present patent application, differentiation is started using $2x10^6$ ES cells/ml if resulting EBs are diluted after 6 h, or 0.1 - $0.5x10^6$ ES cells if the resulting EBs are diluted after 48 h. With this method about 500 - 1000 EBs per ml can be generated, from which about 95 % showed spontaneous beating cardiac cells and express GFP under control of the cardiac α-MHC promotor. If the cardiac cells are purified, typically 1 - 2 cardiomyocytes can be obtained per ES cell originally seeded into the agitation culture.

12. Using the method described in the above cited literature, we inoculated a 250 ml spinner flask with $0.2x10^6$ ES cell per ml and found that using this method only approximately half the number of EBs per ml can be generated, in contrast to the agitation culture of the present invention, differentiation is much lower, yielding only 0.36 cardiomyocytes per ES cell originally seeded into the flask.

13. To illustrate these results, the microphotographs in Exhibit B show a) differentiated EBs on day 10 of a typical agitation culture as described above and claimed in the present patent application, and b) differentiated EBs on day 10 of the spinner flask culture described above. Furthermore, Fig. C shows as a typical result the differentiation into cardiomyocytes, clearly demonstrating the enhanced differentiation when agitating the culture, *i.e.*, using the method of the present invention, compared to the level of differentiation using the "stirring-method."

14. I hereby declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Dr. Silke Schwengberg

Date: Cologne, 2009-09-24

Dr. Silke Schwengberg

CURRICULUM VITAE

Personalia

Name	Dr. Silke Schwengberg
Nationality	German
Date of birth	September 26th 1967
Place of birth	Luenen, Germany

Professional Experience

since 2001	Senior Scientist and Head of Laboratory at Axiogenesis AG, Köln. Development of stem cell based in vitro assays for toxicology, drug discovery, and cardiovascular safety pharmacology
1999 - 2001	Scientist for "Gastrointestinal Pharmacology" at Janssen-Cilag GmbH, branch of Janssen Research Foundation, a Johnson&Johnson company. Development of *in-vitro* and *ex-vivo* assays for intestinal secretion and absorption.

Education

1993 - 1998	Doctorate at the University of Hannover and at the Department of Gastroenterology and Hepatology at Hannover Medical School Title of PhD thesis: "Isolation and functional characterization of human intestinal mast cells "
1991 - 1992	Master thesis at the Department of Medical Microbiology and Immunology at the Ruhr-University Bochum. Title: "Production and characterization of monoclonal anitbodies against antigens of the rat kidney"
1986 - 1991	Studies in biology at the Ruhr-University Bochum Final Examination in Microbiology/Biochemistry, Zoology and Immunology

PUBLICATIONS

Peer review

(1) Marx-Stoelting P, Adriaens E, Ahr HJ, Bremer S, Garthoff B, Gelbke HP, Piersma A, Pellizzer C, Reuter U, Rogiers V, Schenk B, Schwengberg S, Seiler A, Spielmann H, Steemans M, Stedman DB, Vanparys P, Vericat JA, Verwei M, van der Water F, Weimer M, Schwarz M
A review of the implementaiton of the embryonic stem cell test (EST). The report and recommendations of an ECVAM/ReProTect Workshop
Altern Lab Anim 2009 Jul; 37(3):313-28

(2) Sachinidis A, Schwengberg S, Hippler-Altenburg R, Meriappan D, Kamisetti N, Seelig B, Berkessel A, Hescheler J
Identification of small signalling molecules promoting cardiac-specific differentiation of mouse embryonic stem cells.
Cell Physiol Biochem 2006 Dec; 18(6):303-14

(3) Schwengberg S, Bohlen H, Kleinsasser N, Kehe K, Seiss M, Walther U, Hickel R, Reichl FX
In vitro embryotoxicity assessment with dental restorative materials.
J Dent. 2005 Jan; 33(1):49-55.

(4) Bischoff SC, Schwengberg S, Lorentz A, Manns MP, Bektas H, Sann H, Levi-Schaffer F, Shanahan F, Schemann M
Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells.
Neurogastroenterol Motil. 2004 Apr; 16(2):185-93.

(5) Lorentz A, Schwengberg S, Sellge G, Manns MP, Bischoff SC
Human intestinal mast cells are capable of producing different cytokine profiles: role of IgE receptor cross-linking and IL-4.
J Immunol. 2000 Jan 1; 164(1):43-8.

(6) Lorentz A, Schwengberg S, Mierke C, Manns MP, Bischoff SC
Human intestinal mast cells produce IL-5 in vitro upon IgE receptor cross-linking and in vivo in the course of intestinal inflammatory disease.
Eur J Immunol. 1999 May; 29(5):1496-503.

(7) Bischoff SC, Sellge G, Schwengberg S, Lorentz A, Manns MP
Stem cell factor-dependent survival, proliferation and enhanced releasability of purified mature mast cells isolated from human intestinal tissue.
Int Arch Allergy Immunol. 1999 Feb-Apr; 118(2-4):104-7.

(8) Bischoff SC, Lorentz A, Schwengberg S, Weier G, Raab R, Manns MP
Mast cells are an important cellular source of tumour necrosis factor alpha in human intestinal tissue.
Gut. 1999 May; 44(5):643-52.

(9) Bischoff SC, Schwengberg S, Raab R, Manns MP
Functional properties of human intestinal mast cells cultured in a new culture system: enhancement of IgE receptor-dependent mediator release and response to stem cell factor.
J Immunol. 1997 Dec 1; 159(11):5560-7.

(10) Falkenberg FW, Hildebrand H, Lutte L, Schwengberg S, Henke B, Greshake D, Schmidt B, Friederich A, Rinke M, Schluter G, Bomhard E
Urinary antigens as markers of papillary toxicity. I. Identification and characterization of rat kidney papillary antigens with monoclonal antibodies.
Arch Toxicol. 1996; 71(1-2):80-92.

(11) Bischoff SC, Schwengberg S, Wordelmann K, Weimann A, Raab R, Manns MP
Effect of c-kit ligand, stem cell factor, on mediator release by human intestinal mast cells isolated from patients with inflammatory bowel disease and controls.
Gut. 1996 Jan; 38(1):104-14.

(12) Hildebrand H, Falkenberg FW, Schwengberg S, Schlüter G, Bomhard E
Preparation and characterization of a mouse monoclonal antibody against a rat kidney papillary antigen.
Toxicol in vitro 1993 July; 7(4):421-425.

Selected Abstracts (Posters and Talks)

(1) Kettenhofen R, Kolossov E, Ehlich A, Schwengberg S, and Bohlen H
Mouse ES-Cell Derived Cardiomyocytes are a Predictive Tool for Safety Pharmacology when Compared to Human IPS Derived Cardiomyocytes.
World Pharmaceutical Congress 2009, Philadelphia PA, USA

(2) Clarke E, Schwengberg S, Kettenhofen R, Dos Santos G, Bohlen H
Reliable and predictive in vitro assays for myelotoxicity and cardiotoxicity of kinase inhibitors.
SOT 2009, Baltimore, USA

(3) Schwengberg S, Kettenhofen R, Clarke E, Bohlen H
Use of embryonic stem cell derived cardiomyocytes for determination of cardiac toxicity
SPS Annual Meeting 2008, Madison, USA

(4) Schwengberg S and Bohlen H
Pre-Clinical Assessment of cardiac function: Relevance of ES cell derived systems in drug development and toxicology.
World Pharmaceutical Congress 2008, Philadelphia, USA

(5) Schwengberg S, Ehlich A, Marquardt H, Hescheler JH, Bohlen H
Novel reporter gene assay for in-vitro developmental toxicity testing.
SOT 2005, New Orleans, USA

(6) Schwengberg S and Bohlen H
Der embryonale Stammzelltest zum Screening teratogener Wirkungen.
Talk at the Workshop "Novel test methods and their impact for regulatory toxicology" of the study group "Regulatory Toxicology" of the German Society for Pharmacology and Toxicology DGPT, Munich, Germany, October 2002

(7) Osikowska-Evers B, Schwengberg S, Wermelskirchen D
Secretion mediated by activation of guanylate cyclase C (GC-C) by guanylin in T84 cells is largely independent of Cl- efflux.
Digestive Disease Week 2001, Atlanta, USA

(8) Schwengberg S and Wermelskirchen D
The tachykinins Substance P (SP) and Neurokinin A (NK-A) induce intestinal secretion in guinea pig tissue which can be blocked by GR-203040, a NK-1 specific antagonist.
Digestive Disease Week 2001, Atlanta, USA

(9) Schwengberg S, Lorentz A, Manns MP, Bischoff SC
Role of neuropeptides in the regulation of human intestinal mast cells.
Digestive Disease Week 1998, New Orleans, USA

(10) Falkenberg FW, Schwengberg S, Soprani D, Grys B, Sander E, Bomhard E
Demonstration of oscillating damage/recovery events in rat kidneys after daily oral doses of substituted aromatic hydrocarbons.
SOT 1993, New Orleans, USA

Patent Applications

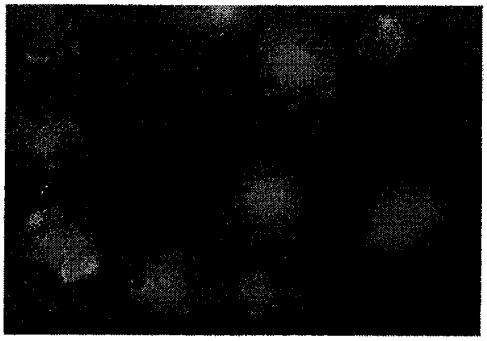
(1) **WO 2005/005662** PCT/EP2004/007529: "Secreted Proteins as Markers for Cell Differentiation"

(2) **WO 2005/005621** PCT/EP2004/007530: "Novel Method for the Preparation of Embryoid Bodies (EBs) and Uses Thereof

(3) **WO 2005/108598** PCT/EP2005/005087 "Assay for Drug Discovery based on in vitro Differentiated Cells "

Figures of Experimental report of Dr. Schwengberg, Axiogenesis AG

i)



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